

# Germination Response of Seeds of *Aconitum grossedentatum* (Ranunculaceae) to Temperature and its Significance to the Expansion of the Distribution

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Germination response of seeds of *Aconitum grossedentatum* to temperature was analyzed and its significance to the distribution of the genus is discussed. The underdeveloped embryo in ripe seeds grew at constant temperatures of 5 and 10°C, but did not grow at a constant temperature of 0°C. Seeds at a constant 5°C germinated gradually, but did not germinate at a constant 10°C. At 10°C embryos became dormant after growing to about 2 mm in length. Stratification at 0°C of seeds incubated at a constant 10°C resulted in synchronous germination. The germination responses to temperature fit well the annual changes in temperature in the habitat of *A. grossedentatum*. It is presumed that seeds need a period of one and a half years for germination in nature. Response of the developing cotyledons to temperature in early spring may be a controlling factor in the spread of the species by seeds.

Key words: *Aconitum grossedentatum*, distribution, dormancy, germination, stratification, temperature

Germination properties in some plant groups have been studied in relation to distribution (e.g., Okagami & Kawai 1982, Bevington 1986, Graves & Taylor 1988, Neuffer & Hurka 1988, Inoue & Washitani 1989, Nishitani & Masuzawa 1996, De Souza *et al.* 1999, Yang *et al.* 1999, Moravcova *et al.* 2001), species diversity (e.g., Frost & Cavers 1975, Jain 1982, Wulff 1988, Hayashi 1990, Bloom *et al.* 1990, Davidson *et al.* 1996, Schuetz 2000), and species conservation (Beigh *et al.* 2002). From these studies, it appears that seeds are well adapted ecophysiologically to germinate in a specific habitat. Baskin & Baskin (1986) reported germination of *Isopyrum biternatum* (Ranunculaceae), a spring ephemeral, to be correlated with annual temperature in the habitat of the species.

Species of *Aconitum* occur mainly in grasslands and in the margins and understory of forests in

the temperate zone. They bloom from late summer to early autumn and disperse ripe seeds in late autumn, in contrast to *Isopyrum biternatum*. The different life histories, that is, differences in timing of seed dispersal, may result in different germination properties. It is presumed that germination in response to temperature restricts the distribution of plants in the temperate zone. Even if seeds are highly viable, they do not germinate well if the germination response does not fit the habitat. Differentiation in germination response may be an important factor in the distribution of plants in the temperate zone.

The aim of this study was to analyze the germination responses of *Aconitum grossedentatum* to various temperatures to see if temperature is a limiting factor in the distribution of the species.

## Materials and Methods

*Aconitum grossedentatum* (Nakai) Nakai (Ranunculaceae) occurs in grasslands and in the margins of temperate forests from central Honshu to the Kinki District, Japan. Ripe seeds of *A. grossedentatum* were collected from the summit of Mt. Ryozen, at about 1000 m elevation, in Shiga Pref., Kinki District, Japan. The summit is a grassland with deciduous perennial plants and shrubs about 0.8-1.5 m tall.

Voucher specimens have been deposited in the herbarium of the Botanical Gardens, Faculty of Science, Osaka City University.

Preliminary tests indicated that seeds germinated equally well under light and dark conditions, thus, this study was conducted only in the light. The photoperiod at all temperatures was 12 hr. Freshly collected seeds were incubated in distilled water in 9 cm glass laboratory dishes, and water was added as needed during the experiments. Two replicates of 100 seeds were tested in each treatment. Germination tests at 0°C were carried out in the freezing compartment of a refrigerator. Tests at 5°C and 10°C were done in a temperature controlled incubator (Nihon Ikaki co., TG-100-AD). Responses to 15° and 20°C were also attempted, but failed because fungi destroyed many seeds. All temperatures were controlled to within  $\pm 1^\circ\text{C}$ . Seed germination was checked at weekly intervals for more than 15 months. The timing of germination was judged by radicle protrusion. The germination rate was calculated from the mean of both dishes.

For the measurement of embryo length, additional dishes with several hundreds seeds were also incubated under the same conditions. The length of the embryo was measured using 10-20 seeds fixed in 70% EtOH. The embryos were removed from the seeds and their length was measured under a microscope. The mean values  $\pm$  standard error (SE) were calculated. After seed germination began, mean embryo size was estimated using the following

equation.

$$\text{Mean value (mm)} = \{ \text{TOV} \times (1 - \text{GR}) + 3.25 \times \text{GR} \times N \} / N$$

When, TOV; total sum of observed value of embryo size, GR; germination rate, N; seed number observed. The value of 3.25 was employed from a seed size of about 3.00 mm in length.

The annual change in monthly mean temperature at the summit of Mt. Ryozen was estimated from data obtained at the Hikone Meteorological Observatory, at 87.3 m above sea level, (Chronological Scientific Tables 1985), which is situated near the locality, by assuming a decrease in temperature of  $0.55^\circ\text{C}$  per 100 m increase in elevation.

## Results

Figure 1 shows the germination rate of the seeds of *Aconitum grossedentatum* at constant 0°, 5° and 10°C. There were clear differences among the conditions. At constant 0°C, about seven percent of the seeds germinated, but the embryos did not grow when held at a constant 0°C throughout the test (Fig. 2). The immature embryos were about 0.5 mm in length, therefore underdeveloped (sensu

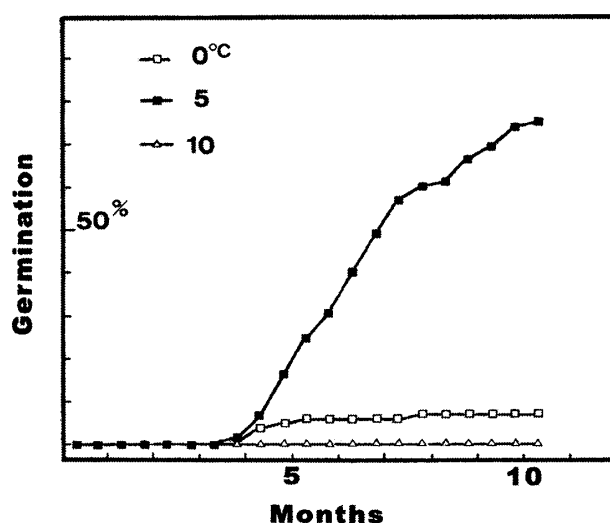


FIG. 1. Seed germination of *Aconitum grossedentatum* at constant 0, 5 and  $10^\circ\text{C}$ .

Grushvitzky 1967). The embryo size was larger than given in the report by Tamura & Mizumoto (1972).

Embryos grew when the temperature reached 5 or 10°C. At a constant 5°C, the seeds germinated after four months, and the germination rate reached 50 % after 3-4 months from the first germination. The final germination rate was ca. 80 % (Fig. 1). The delay in germination was due to the long after-ripening period of embryo (Fig. 2). Embryos at a constant 5°C developed gradually, some faster than others, and some remained at a very early stage of development. In all cases, they developed continually until germination.

Seeds incubated at a constant 10°C did not germinate (Fig. 1). The embryos developed more or less synchronously until their lengths reached about

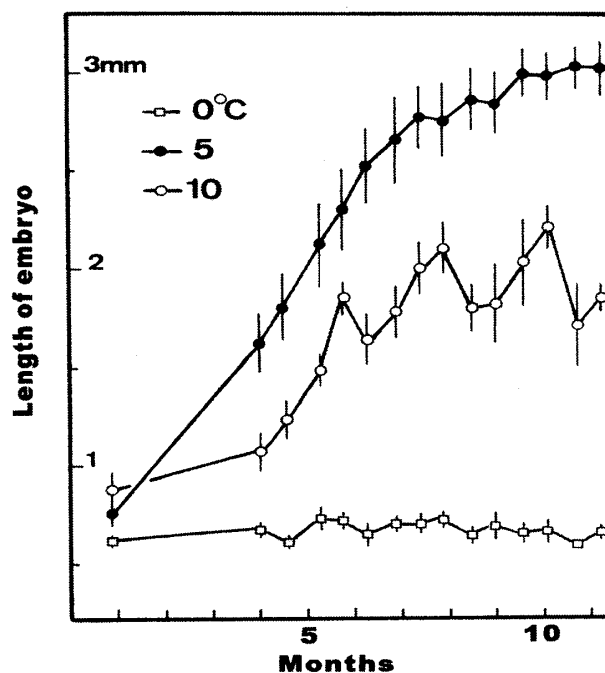


FIG. 2. Growth of embryos of *Aconitum grossedentatum* (mean  $\pm$  SE) at constant 0, 5 and 10°C.

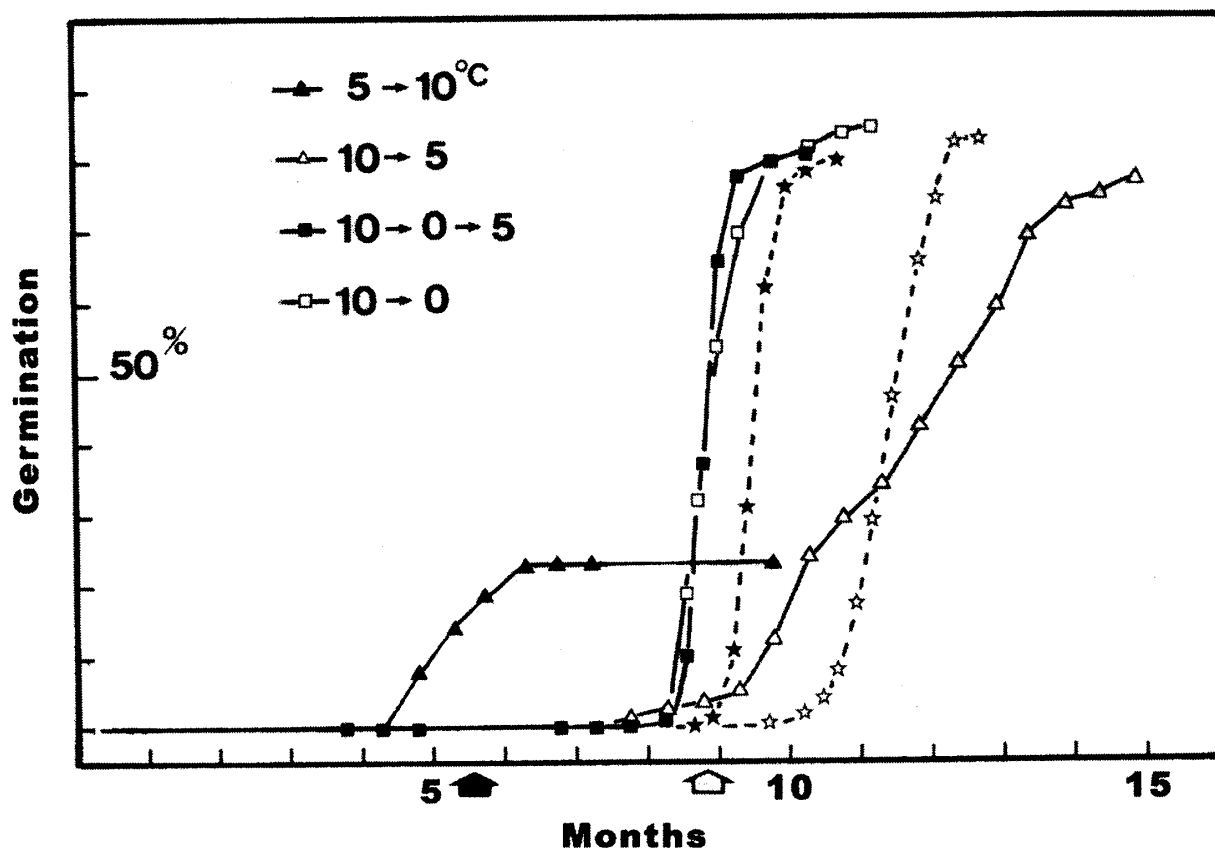


FIG. 3. Seed germination of *Aconitum grossedentatum* after transfer to different temperature conditions (5→10, 10→5, 10→0 and 10→0→5°C). Seeds germinated synchronously after being transferred to 0°C conditions after being held dormant at 10°C. Expansion of cotyledons of seeds transferred to 5°C (white arrow) was accelerated. Stars and broken lines indicate percent of seedlings in which cotyledons expanded (solid stars; 10→0→5°C, open stars; 10→0°C)

2 mm after about six to eight months, and then they stopped developing (Fig. 2). Seeds were dormant at 10°C. This after-ripening dormancy of the embryo was the reason the seeds did not germinate at 10°C, contrary to embryos at 0°C, which remained underdeveloped throughout the life of the experiment. The seeds at a constant 5°C suddenly became dormant when the temperature was changed to 10°C (Fig. 3).

The dormancy of seeds incubated at a constant 10°C for about 6 months was effectively broken by stratification at 0 or 5°C for a few months (Fig. 3). Seeds stratified at 5°C germinated gradually, as in the constant 5°C conditions. At 0°C, the seeds germinated synchronously, and the germination rate

reached 80–88 % after one month from the first seed germination. Further, the change in temperature from 0 to 5°C after three months from the first alteration (Fig. 3, white arrow) accelerated the opening of the cotyledons. The effect of stratification at 0°C became clear when the period at 0°C was more than one month, but unclear for periods of less than two weeks (Fig. 4).

## Discussion

The germination scenario under natural conditions on Mt. Ryozen is presumed to be as shown in Fig. 5. After the seeds are dispersed in late autumn, underdeveloped embryos grow slightly until the temperature becomes low. The embryos can not grow during winter. In the following spring when temperatures exceed the minimum of 5°C, the embryos resume development until after several months they reach maturity. At that time, the seeds do not germinate because they remain dormant at temperature higher than 10°C. Low temperatures, at 0°C, for a few months the next winter induces vernalization of the seeds. Early the next spring when temperatures again exceed by 5°C, the cotyledons quickly expand. It is presumed that seeds in the natural habitat on Mt. Ryozen will germinate in the spring of their second year. The germination response of *Aconitum grossedentatum* to temperature appears to be well adapted to the annual temperature cycle of the habitat. Synchronous germination is caused by certain process, i.e., dormancy of the after-ripening embryo below 10°C and following stratification at 0°C for a few months.

The expansion of the cotyledons in the natural environment on Mt. Ryozen is timed to coincide with various seasonal stages throughout the year. At first, the embryos develop to maturity from May to October or November, then the seeds go dormant. Dormancy is effectively broken when temperatures drop to 0°C. The period of dormancy is flexible. The period of stratification at 0°C is also flexible,

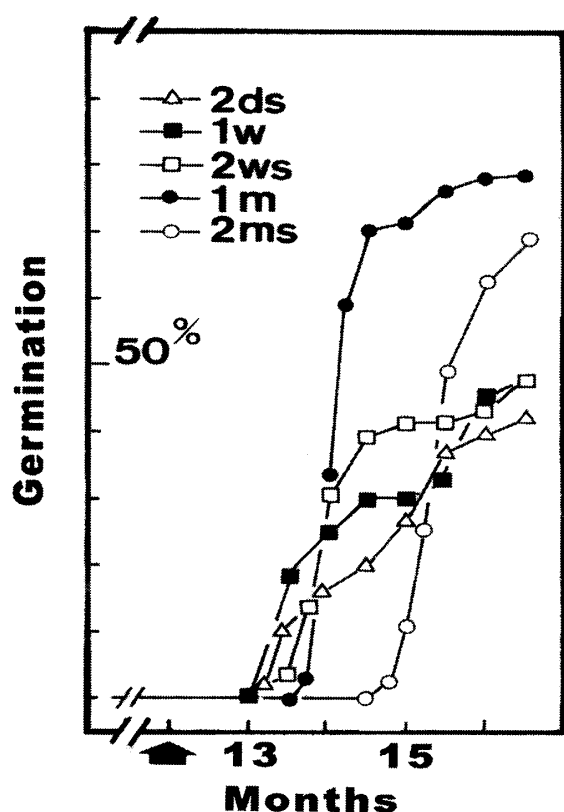


FIG. 4. Effects of length of stratification period at 0°C (2 days, 1 week, 2 weeks, 1 month and 2 months) on seeds of *Aconitum grossedentatum* incubated at 10°C for one year. After stratification seeds were transferred to 5°C. Black arrow indicates time when seeds were transferred to 0°C. Seeds stratified for less than two weeks germinated gradually, like those under constant 5°C. Stratification for one month at least 0°C is necessary for synchronous germination. Stratification for two months at 0°C delayed germination.

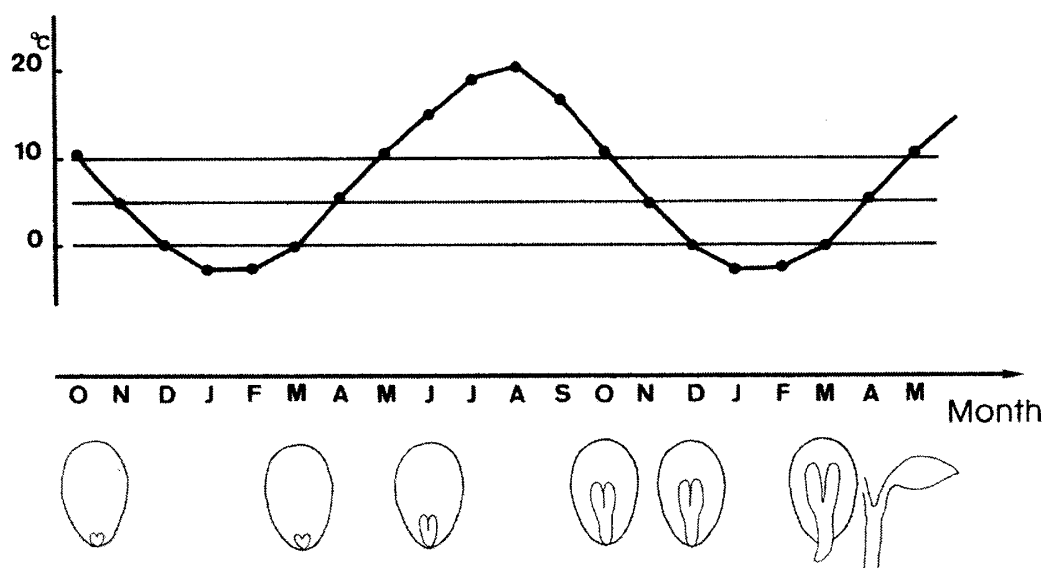


FIG. 5. Calculated annual monthly mean temperature in nature on Mt. Ryozen. Schematic drawing of embryo development estimated. Ripe seeds were dispersed in late October while embryos were still underdeveloped. Germination requires a period of one and a half years.

i.e., more than one month (Fig. 4). Finally, the expansion of the cotyledons is accelerated by temperatures above 5°C (Fig. 3). The period during which the cotyledons remain within the seed may be flexible (Fig. 4). I concluded that the seeds of *Aconitum grossedentatum* on Mt. Ryozen can easily adjust the timing of the expansion of their cotyledons to early spring wherever they occur within the range of environmental variation as mentioned above.

The rapid expansion of the cotyledons in the early spring is considered to be an important factor for seedling survival in an environment where surrounding plants also being growth. By beginning growth early, seedlings are able to receive sufficient sunlight before being shaded by neighboring plants. I have concluded that the conditions for survival of seedlings of *Aconitum grossedentatum* on Mt. Ryozen are: high temperature after dispersal, a minimum of 10°C, for more than six months for development of the embryo, low temperature of 0°C for more than one month, and then above 5°C for more than one month. This combination of temperatures may be the main factors limiting the distribution of *A. grossedentatum*.

Baskin & Baskin (1986) report that *Isopyrum biternatum* (Ranunculaceae), a spring ephemeral, also has an underdeveloped embryo, but the seeds are not dormant and germinate in the autumn, thereby differing from the situation in *Aconitum grossedentatum*. Their germination is accelerated by pre-incubation at high temperature (alternating 12/12h regimes of 30/15°C). Thus, seeds with an underdeveloped embryo show various germination patterns (Baskin & Baskin, 1984).

According to Beigh *et al.* (2002), the seeds of *Aconitum heterophyllum* from natural populations in the northwest Himalaya germinate at the onset of spring (March-April) after dormancy during the winter. The most effective treatment for inducing seed germination in *A. heterophyllum* is pre-chilling for 30 or 90 days. The seeds of *A. heterophyllum* ripen in autumn, the same as *A. grossedentatum*, but differ in their germination pattern from *A. grossedentatum*. The germination of *A. heterophyllum* takes only half a year. They did not describe the embryo condition in ripened seeds in their report, but differences in germination patterns within *Aconitum* shows the adaptation to the environment that has made the genus so successful in

eastern Asia (Graves & Taylor 1988, Nishitani & Masuzawa 1996).

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